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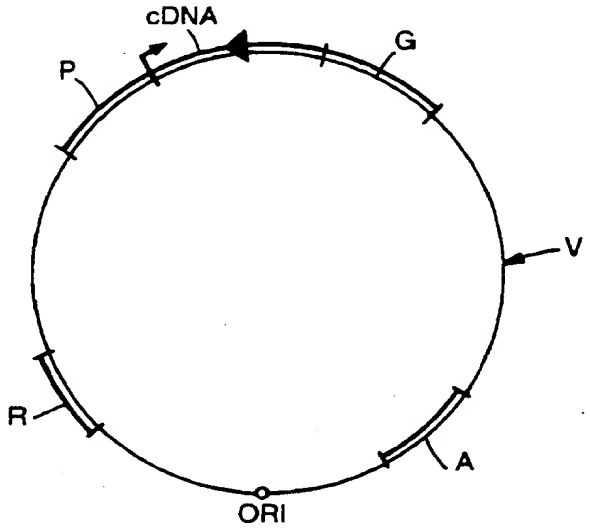
(54) Antisense vector

(57) A vector (V) comprises a gene (cDNA) fused to a gene (G) for an overt phenotypic characteristic, along with a promoter sequence (P) oriented such that the gene (cDNA) is transcribed into antisense RNA. If cells are transfected with the vector (V), and the vector is transcribed, then antisense-RNA fused to mRNA for the overt phenotypic characteristic is formed. If the antisense-RNA hybridises with the cell's sense mRNA for a target protein, then expression of the overt phenotypic characteristic will be suppressed (as too is expression of the target protein). Hence cells exhibiting this antisense effect can readily be identified.



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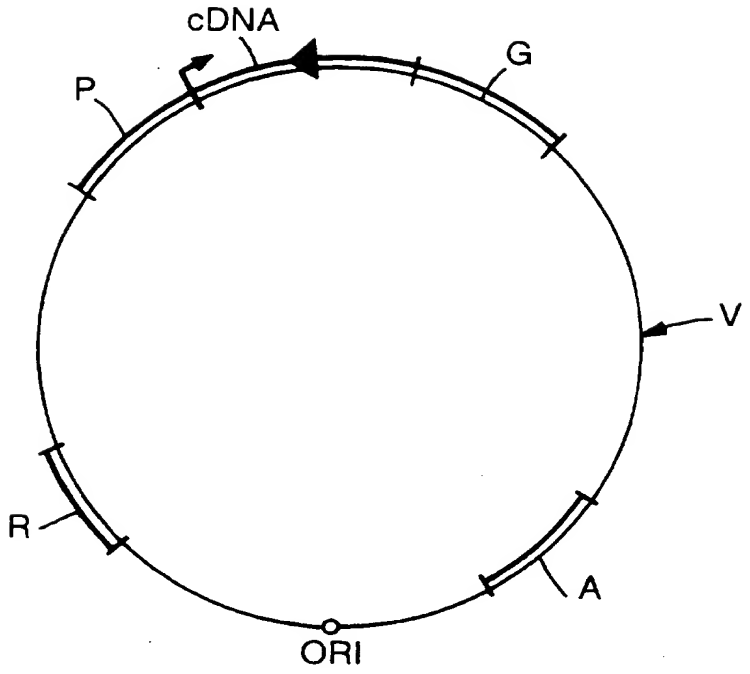
At least one drawing originally filed was informal and the print r produced here is taken from a later filed formal copy.



Antisense Vector

This invention relates to a vector comprising a gene for antisense RNA, and a method for introducing antisense RNA into a cell.

The process by which biological cells make proteins involves two stages. The sequence of amino acids required to make the protein is specified in a gene, which consists of two strands of DNA, each amino acid being encoded by a codon of three adjacent nucleotide bases in one of the DNA strands; the other strand of DNA has the complementary sequence of nucleotide bases. In the first stage the sequence of bases in the one DNA strand is transcribed, in accordance with cis-acting control sequences, to form a complementary strand of mRNA. The mRNA is then transported out of the cell nucleus into the cytoplasm, where, as the second stage, the mRNA is translated at a ribosome to form the specified protein. This process can be inhibited in a cell by the presence of antisense RNA. The term antisense RNA means an RNA molecule whose sequence of bases is complementary to a sequence of bases in the mRNA in question, so that each base (or the majority of bases) in the antisense RNA (read in the 3' to 5' direction) is capable of binding to the corresponding base in the mRNA (read in the 5' to 3' direction) ; guanine (G) binds to cytosine (C), adenine (A) binds to uracil (U). The antisense RNA binds to the mRNA and prevents it being transcribed. Thus the provision of the appropriate antisense RNA in a cell inhibits the production of a specific protein. This procedure may be used in in vitro studies of gene function, to find if the inhibition of production of a specific protein (by antisense gene ablation) inhibits a cellular function. The procedure may also be applied to the in vitro study of human

diseases, and potentially as a method of therapeutically blocking the expression of a harmful gene.

One way in which antisense RNA may be provided in a cell is to introduce into the cell a gene to make antisense RNA (along with the requisite transcription signals), by transfecting target cells with a cloned plasmid vector containing the antisense sequence under the control of a eukaryotic promotor. The vector may also contain a suitable marker gene to enable those cells into which the vector has been incorporated to be readily selected. For example the marker gene might express resistance to a cytotoxic drug. However, for reasons which are not at present fully understood, of those cells where the vector has been incorporated only a small proportion also exhibit the expected inhibition of the specific protein.

According to the present invention there is provided a vector comprising a gene for an antisense RNA, the antisense RNA gene being fused to a gene for an overt phenotypic characteristic, such that if the vector is incorporated in a cell and is transcribed the resulting RNA comprises the antisense RNA fused to the mRNA for the overt phenotypic characteristic.

If such a vector is used and the fused gene is transcribed, then if the antisense RNA binds to the complementary sense mRNA not only is production of the corresponding protein inhibited, but also the overt phenotypic characteristic will not be expressed. The inhibition of the target gene expression may occur as a result of the destruction of the double-stranded RNA hybrid by cell enzymes, or by the inhibition of the translation of the target mRNA by steric effects; and expression of the overt phenotypic characteristic is also

inhibited. Hence the transfected cells can easily be screened to find those in which antisense gene ablation has occurred. In one example the overt phenotypic characteristic is green fluorescent protein.

The vector also comprises a marker gene for a readily selectable characteristic, such as resistance to a cytotoxic drug (e.g. hygromycin).

The invention will now be further and more particularly described by way of example only, and with reference to the accompanying drawing.

It is desired to find the role of a specific target protein in a human cell culture. Copy DNA (cDNA) is made from the mRNA corresponding to the protein; this cDNA is then cloned in a bacterial culture.

Referring to the drawing, the cDNA (or a cDNA fragment) corresponding to the target protein is then ligated in the antisense direction to a eukaryotic promoter sequence P, and the other end of the cDNA is fused to an overt phenotypic gene G which in this example is the gene for green fluorescent protein (gfp). Plasmid vectors V comprising this promoter/antisense-cDNA/gfp gene and also a marker gene R for resistance to a drug such as hygromycin (which kills mammalian cells) are constructed. In this example the plasmid V also includes an origin of replication (ori) and a gene A for resistance to ampicillin so the plasmid could if desired be replicated in a bacterial culture.

The plasmid vectors V are then transfected into a suspension or a mono layer of mammalian cells by conventional methods for example liposome-mediated transfection. The cell culture is then incubated in the

presence of hygromycin, so that only those cells in which the vector V is stably integrated into the cell genome survive. Typically in less than 0.01% of the cells is the vector V integrated into the genome so that the cells survive. Hence from a starting culture of 10^6 cells perhaps only 100 colonies of hygromycin-resistant cells may be obtained. The surviving cells are then sorted optically, according to whether or not they fluoresce, indicating that the gfp gene is being expressed. Those which do not fluoresce are those in which the antisense RNA is hybridising with the target mRNA, and so these cells can be expected to express lower amounts of the target protein whose role is being investigated.

The cells can alternatively be sorted by performing these two tests in the reverse order : first identifying those cells which fluoresce, and then incubating those which don't fluoresce in a medium containing hygromycin to kill those cells in which the plasmid vector V is not incorporated. It would be expected that all those cells which do fluoresce would also survive hygromycin treatment, but their antisense RNA is evidently not hybridising with the target mRNA.

It will be appreciated that the gene G for the overt phenotypical characteristic might correspond to a different characteristic. For example it might be a gene for an enzyme such as secreted alkaline phosphatase. In this case it is not feasible to test individual cells to find if the gene G is being expressed. Instead the transfected cells would be cultured in a medium containing hygromycin. Colonies of cells which grow in this medium can then be assayed for whether or not they secrete the enzyme; if they don't secrete the enzyme they

can be expected to exhibit the desired antisense effect and so to express lower amounts of the target protein.

It will also be appreciated that a plasmid of the invention may differ from that described above, for example it might incorporate a gene for resistance to a different drug, such as G-418 which is an aminoglycosidic antibiotic which kills eukaryotic cells which do not express the gene for aminoglycoside phosphotransferase. Equally a plasmid might not include the ori and ampicillin-resistance features, if it was not desired to clone it in bacterial cultures. The cDNA gene might correspond to the complete genetic sequence of the mRNA for the target protein, or might correspond to only a part of that genetic sequence.

Claims

1. A vector comprising a gene for an antisense RNA, the antisense RNA gene being fused to a gene for an overt phenotypic characteristic, such that if the vector is incorporated in a cell and is transcribed the resulting RNA comprises the antisense RNA fused to the mRNA for the overt phenotypic characteristic.

2. A vector as claimed in Claim 1 where the overt phenotypic characteristic is green fluorescent protein.

3. A vector as claimed in Claim 1 or Claim 2 also comprising a marker gene for a readily selectable characteristic.

4. A vector substantially as hereinbefore described with reference to and as shown in the accompanying drawing.

5. The use of a vector as claimed in any one of the preceding Claims to transfect cells.

6. A method of introducing antisense RNA into a cell by transfecting the cell with a vector as claimed in any one of Claims 1 to 4.

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The Patent Office

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Claims searched:	1 to 6	**Date of search:**	26 February 1997

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.O): C3H (HB7P)

Int Cl (Ed.6): C12N 15/11

Other: ONLINE: DIALOG/BIOTECH, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
	No relevant documents found	-

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.